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USE OF CHLOROPHYLL FLUORESCENCE PARAMETERS TO ASSESS DROUGHT TOLERANCE OF COCONUT VARIETIES

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ABSTRACT

Chlorophyll fluorescence analysis as a measure of photosynthesis is increasingly used in eco-physiological studies. It is particularly useful in investigations on the photosynthetic performance of plants in stressful environments. Chlorophyll fluorescence, which can be measured rapidly and conveniently, provides insights into a plant's ability to tolerate environmental stresses such as those of a drought. Damage to the photosynthetic apparatus due to moisture or heat stress, which would reduce photosynthetic assimilation and eventually the yield, can be detected at a very early stage of the drought. This technique is widely used to identify stress tolerant plants and crop varieties. However, this technique has not been utilized to identify putative drought tolerant coconut varieties. Hence, the objective of this study was to set out the basic principles of the technique, discuss its applications in eco-physiological studies, and evaluate its potential to screen coconut varieties for drought tolerance.

San Ramon, a tall form of coconut introduced to Sri Lanka few decades ago and a few other local tall accessions in the germplasm conservation blocks in the Coconut Research Institute of Sri Lanka appeared to possess a photosynthetic apparatus which was clearly more tolerant to moisture stress conditions than the other accessions. *SRR, SRG, SA, CL PI & PW* were the most drought tolerant of the 40 genotypes tested. The results generated by using F_v/F_m, were consistent with results of other important parameters such as the rate of photosynthesis and water use efficiency. Even the basic parameters of chlorophyll fluorescence, for instance the maximum quantum yield of photosystem II (F_v/F_m) measured on dark-adapted coconut leaves showed promise as a selection criterion in future screening programmes due to its quick, non-invasive and reliable nature. Similarities of fluorescence parameters observed between seedlings and adult palms enables fairly confident predictions on the performance of adult palms, based on studies with seedlings. In general, chlorophyll fluorescence appears as a promising tool for screening coconut palms for their drought tolerance.

Key words: Coconut, drought tolerance, chlorophyll fluorescence, photosynthesis, F_v/F_m

INTRODUCTION

In recent times the chlorophyll fluorescence technique has been increasingly used in eco-physiological studies with important crops and plant species. Investigations on the photosynthetic performance of plants in stressful environments are now deemed incomplete without information on chlorophyll fluorescence (Maxwell and Johnson, 2000) especially, since the introduction of a variety of sophisticated, highly user-friendly, portable chlorophyll fluorometers. Despite the simplicity of taking measurements, the theoretical aspects of the technique and the interpretation of data are complex and controversial. Although the potential for using the chlorophyll fluorescence technique to screen plant species for putative drought tolerance has been established, it has not been tested for coconut. Hence, this paper discusses the basic principles of the chlorophyll fluorescence technique, its eco-physiological applications and limitations, and proceeds to evaluate the potential for using the technique to screen coconut varieties for drought tolerance. The results of preliminary studies with coconut seedlings under plant house conditions and adult palms under field conditions are presented.

Chlorophyll fluorescence – the underlying principles

Each quantum of light absorbed by a chlorophyll molecule raises an electron from ground state to an excited state. Part of this light energy, absorbed by chlorophyll molecules in the leaf, is used to drive the photosynthetic process (photochemistry); the excess energy is either dissipated as heat, or re-emitted as red light, which is called chlorophyll fluorescence. These three processes occur simultaneously; an increase in the efficiency of one process will reduce the yield of the other two. It is therefore possible to derive information on the efficiency of photochemistry and heat dissipation by measuring the yield of chlorophyll fluorescence.

The chlorophyll fluorescence spectrum is clearly different from that of absorbed light; the fluorescence peak has a longer wavelength than that of absorbed light. Measuring chlorophyll fluorescence is therefore relatively easy, even though total chlorophyll fluorescence is only 1-2% of total light absorbed. The fluorescence yield can be quantified by exposing a leaf to a defined wavelength and measuring the amount of light re-emitted at a longer wavelength. The advent of a 'modulated' measuring system (a modification to the basic measuring device), was pivotal in revolutionizing the application of chlorophyll fluorescence (Quick and Horton, 1984). Here, the light source used to induce fluorescence is 'modulated' (switched

on and off at a high frequency) and the detector is adjusted to detect only the fluorescence excited by the measuring light. This enabled measuring the relative yield of fluorescence in the presence of background light, even under full sunlight in the field.

Kautsky effect

In 1960, Kautsky and co-workers first observed that transferring photosynthetic material from dark into the light increased the yield of chlorophyll fluorescence for about one second (Kautsky *et al.*, 1960). This increase was explained later as being due to the reduction of electron acceptors in the photosynthetic pathway, down stream of photosystem II (PSII), more precisely plastoquinone, Q_A. When PSII absorbs light and Q_A accepts an electron, it cannot accept another electron until it passes the electron to the subsequent electron carrier Q_B. Until then, the reaction centre is said to be 'closed'. The closing of reaction centres leads to an overall reduction in the efficiency of photochemistry and a simultaneous increase in the yield of fluorescence. The magnitude of these changes, at any given time, is related to the proportion of closed centres.

When a dark-adapted leaf is transferred to light, PSII reaction centres are progressively closed giving rise to an increase of chlorophyll fluorescence for a very brief period. However, the fluorescence level drops over a period of few minutes and this phenomenon is termed fluorescence quenching. There are two processes that contribute to fluorescence quenching. Firstly, the rate at which electrons are transported away from PSII is increased due to the light-induced activation of enzymes involved in carbon metabolism and opening of stomata. This is 'photochemical quenching'. Secondly, the efficiency of energy conversion to heat is increased simultaneously and this is termed 'non-photochemical quenching (NPQ). These two processes attain a more or less steady-state condition within about 15-20 minutes, depending on the plant species (Maxwell and Johnson, 2000).

The clear separation of the photochemical and non-photochemical components of quenching is important to extract information on the photosynthetic performance of a plant by measuring chlorophyll fluorescence. The fluorescence yield is estimated wlth one component process operating and the other 'switched off'. The common practice is to switch off the photochemical component. Although addition of the chemical Diuron (DCMU) inhibits PSII and reduces photochemistry to zero, it is not an appropriate method from a physiological and practical point of view. On the other hand, the 'Light doubling technique' i.e. giving

a high intensity, short duration flash of light, which momentarily reduces photochemical quenching to zero, is non-destructive and more convenient (Bradbury and Baker, 1981). In the absence of any photochemical quenching the fluorescence yield reaches the maximum attainable (maximum fluorescence F_m). Comparing this value (F_m) with the steady-state yield of fluorescence in the light (F_t) and the yield of fluorescence in the absence of photosynthetic light (F_o) gives information on the efficiency of photochemical quenching and also the performance of PSII (Maxwell and Johnson, 2000). Although the levels of F_m are subjected to change with changes in heat dissipation (non-photochemical quenching), it is not possible to totally inhibit heat dissipation and measure the yield of chlorophyll fluorescence in the absence of non-photochemical quenching. Therefore, all estimates of non-photochemical quenching have to be taken relative to some dark-adapted point termed as F^o_m. Therefore, it is important to take a dark-adapted, non-stressed reference point, which has to be a pre-dawn value in field experiments.

Some parameters are referred to by different terminology in the literature and this may cause some confusion. However, the general calculation of fluorescence parameters can be explained by reference to a typical experimental trace as shown in Fig. 1, reproduced from Maxwell and Johnson (2000). The measurement is initiated by switching on the measuring light, giving a measure of the minimal level of fluorescence (F_o). A saturating pulse of light is then applied which allows the measurement of F_m in the dark-adapted state (F^o_m). Then, an actinic (photosynthetic) light is applied with further saturating flashes at appropriate intervals in which the maximum fluorescence in the light is measured (F'_m). The fluorescence immediately prior to the flash is termed steady-state fluorescence (F_t) after which actinic light is removed with simultaneous application of far-red light (wavelength >680 nm) which completely nullifies the effect of actinic light and allows measuring F'_o. This far-red light is important to ensure that all PSII reaction centres open rapidly after the end of illumination.

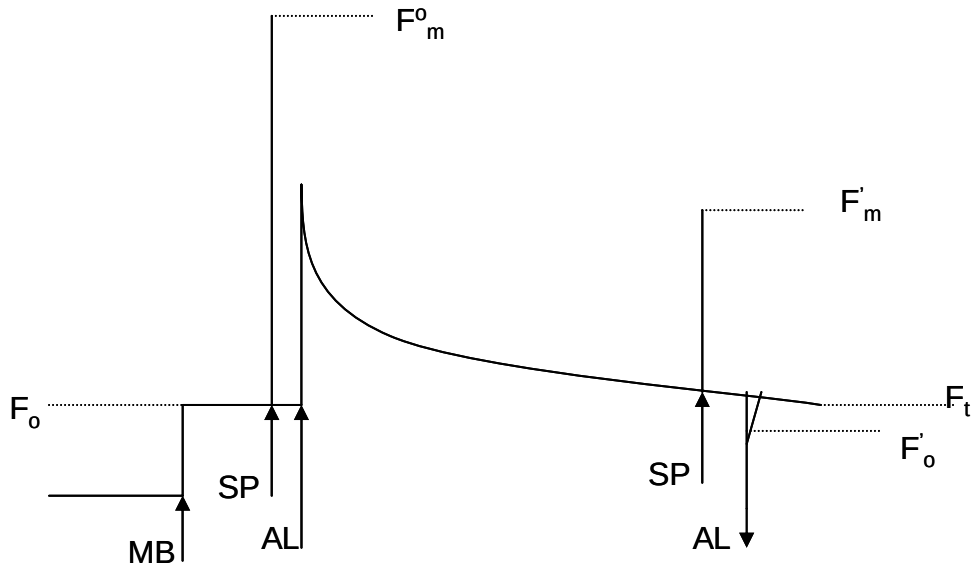

Fig. 1: Sequence of a typical fluorescence trace. A measuring light is switched on (MB) and the zero fluorescence level is measured (F_o). Application of saturating flash of light (SP) allows the measurement of maximum fluorescence level (F^o_m). A light to drive photosynthesis (AL) is then applied. After a period of time, another saturating light flash (SP) allows the maximum fluorescence in the light (F'_m) to be measured. The level of fluorescence immediately before the saturating flash is termed F_t. Turning off the actinic light (AL) typically in the presence of far-red light allows zero level fluorescence in the light to be estimated (From Maxwell and Johnson, 2000).

Important parameters

Photochemical processes

One of the important parameters that can be measured is the efficiency (or the quantum yield) of Photosystem II photochemistry (Φ_{PSII}) which, measures the proportion of the light absorbed by chlorophyll associated with PSII and used in photochemistry as shown below (Genty *et al.*, 1989). This provides a measure of the rate of linear electron transport which is an indication of the overall photosynthesis. Strong linear relationship has been observed between Φ_{PSII} and the efficiency of carbon fixation under laboratory conditions but some discrepancies may occur under stress

conditions due to changes in the rate of photorespiration (Fryer *et al.*, 1998). The rate of linear electron transport (*J*) can be calculated using Φ_{PSII} as shown below (Genty *et al.*, 1989):

$\Phi_{PSII} = (F'_m - F_t) / F'_m$
$J = \Phi_{PSII}$ x PFDa x (0.5)

where, PFDa is absorbed light (μmol photon m^{-2} s^{-1}) and 0.5 is a factor that accounts for the partitioning of energy between PSII and PSI. Provided that absorption of light is constant, the relative changes of *J* between two leaves can be compared simply by multiplying Φ_{PSII} by incident light.

Photochemical quenching (*qP*) is calculated as follows:

$qP = (F'_m - F_t) / (F'_m - F'_o)$

While Φ_{PSII} gives the proportion of absorbed energy used in photochemistry, *qP* provides an indication of the proportion of PSII reaction centres that are open, and therefore, (1 - *qP)* gives the proportion of centres that are closed, which is sometimes termed as 'excitation pressure on PSII (Maxwell *et al.*, 1994).

Φ_{PSII} and *qP* are interrelated by a third parameter F_v/F_m (as given below) which, is a measure of the intrinsic (or maximum) efficiency of PSII (i.e. the quantum efficiency if all PSII centres were open).

$F_v/F_m = (F_m - F_o) / F_m = \Phi_{PSII} / qP$

While Φ_{PSII} relates to achieved efficiency, *qP* and F_v/F_m provide information about the underlying processes which have altered efficiency. While changes in *qP* indicates the closure of reaction centres, resulting from a saturation of photosynthesis by light, a change in F_v/F_m shows a change in the efficiency of non-photochemical quenching. The potential quantum efficiency of PSII represented by the dark-adapted values of F_v/F_m, is a sensitive indicator of plant photosynthetic performance. Optimal values of F_v/F_m are around 0.83 for most plant species (Maxwell and Johnson, 2000; Bjorkman and Demmig, 1987). When plants are exposed to stress, F_v/F_m values would be lower than optimal, indicating the phenomenon of photoinhibition (Maxwell and Johnson, 2000).

Non-photochemical processes

The dark-adaptation of a full night and 24 hour period is generally used under laboratory conditions to measure the dark-adapted values of F_m which is important in quantifying non-photochemical quenching. Moreover, it is assumed that the plant material is not exposed to any stress prior to the start of the experiment. This ensures the obtaining of the reference level of F_m in which photochemical efficiency is at its maximum and heat dissipation is at a minimum. The pre-dawn values of F_m are used as a reference in field experiments. The ratio of the change in F_m to the final value of $F_{m,}$ is the frequently used method for quantifying non-photochemical quenching as given below (Bilger and Bjorkman, 1990).

$NPQ = (F^o_m - F^{'}_m) / F^{'}_m$

NPQ is linearly related to heat dissipation and could range from zero to infinity. Typical values for a plant are in the range 0.5 to 3.5 at saturating light intensities (Maxwell and Johnson, 2000).

Chlorophyll fluorescence - applications in eco-physiology

In general, chlorophyll fluorescence provides information on how the light energy absorbed by chlorophyll impacts on PSII – the extent to which PSII uses the energy and the extent to which PSII is damaged by excess light (Maxwell and Johnson, 2000). The flow of electrons through PSII is indicative of the overall rate of photosynthesis, and this enables a rapid estimation of photosynthetic performance, under conditions where other methods would fail. Moreover, PSII is the most vulnerable part of the photosynthetic apparatus to light-induced damage and this damage is the first manifestation of stress in a leaf. Although these experiments of chlorophyll fluorescence requires very careful designing and are generally coupled with other techniques such as gas exchange measurements, it provides quick but precise estimates of photosynthetic performance even under initial stress conditions.

An advantage of chlorophyll fluorescence is that it provides a measure of photosynthesis. Chlorophyll fluorescence can be converted into a relative rate of linear electron transport. Photosynthesis is the gross rate of carbon fixation. PSII electron transport and CO_2 fixation correlate well under laboratory conditions (Genty *et al.*, 1989; Edwards and Baker, 1993). However, the relationship is obscure under field conditions due to competing processes such as photorespiration, nitrogen metabolism and

electron donation to oxygen (Mehler reaction). Furthermore, the heterogeneity between leaves, especially when grown in different microclimates, makes comparisons less precise. Hence, gas exchange measurements still remain as the core parameter in eco-physiology. Despite above limitations Φ_{PSII} provides useful information on photosynthetic performance in the field with rapid and minimally invasive measurements. Hence, the method has been used successfully in varietal comparisons in very different plant species such as rice (Murchie *et al.*, 1999) and loblolly pines trees (Hymus *et al.*, 1999).

The linear relationship observed between quantum yield of CO_2 fixation and Φ_{PSII} in experiments with simultaneous measurements of CO_2 assimilation and Φ_{PSII} under non-photorespiratory conditions (elevated CO_2 or 1-2% O_2) at different light intensities provide a way to estimate the extent of photorespiration *in vivo* (Epron *et al.*, 1995). This protocol has been used in different plant species to evaluate the significance of photorespiration as a photoprotective mechanism during drought stress (Cornic and Briantais, 1991; Valentini *et al.*, 1995). Meyer and Genty (1998) using high resolution images of Φ_{PSII}, proved that stomatal closure is the primary effect of drought stress which in turn leads to the reduction in intercellular CO_2 concentration (C_i) and thus limits carboxylation. Moreover, Fryer *et al.*, (1998) used fluorescence analysis to understand the effect of low and high temperatures on the photosynthetic process.

Most importantly, chlorophyll fluorescence gives insights into the ability of a plant to tolerate environmental stresses and also the extent to which those stresses damaged the photosynthetic apparatus (Maxwell and Johnson, 2000). Diurnal measurements of chlorophyll fluorescence in different plant species, using portable fluorometers developed recently, have provided a wealth of information on *NPQ*, electron transport rates, quantum efficiency and the extent of photorespiration in response to light, temperature and other environmental stresses (Bilger *et al.*, 1995). Gamon and Pearcy (1989) observed a decrease in F_v/F_m and an increase in F_o which indicated photo inhibitory damage in response to high temperature. Similar effects were also observed for low temperature (Groom and Baker, 1992), high PFD (Ogren and Sjostrom, 1990) and water stress (Epron *et al.*, 1992). With the recent improvements in the technology, changes in F_v/F_m and F_o appear to be valid, reliable diagnostic indicators of photo inhibition and are being widely used (Valladares and Pearcy, 1997; Hakam *et al.*, 2000; Percival and Sheriffs, 2002). For example, a study conducted by Baker and Adams (1997) on a cactus species revealed that cladodes that received high

PFD contain a large xanthophyll cycle pool with de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin which indicated a high *NPQ*.

Chlorophyll fluorescence – as a tool for screening coconut varieties for drought tolerance

MATERIALS AND METHODS

Two experiments, one in the plant house and the other in the field, were carried out to evaluate the potential for using chlorophyll fluorescence to identify putative drought tolerant coconut genotypes. The plant house experiment was conducted with seedlings of four genotypes grown in pots under controlled environment. The field experiment evaluated adult palms from 40 forms and accessions (form/acc).

Plant house experiment

The four coconut forms (in parenthesis) selected for the plant house experiment represented the main 'varieties' of coconut: var. *typica*, or 'talls' (Ambakelle Special, *AS*); var. *nana* or 'dwarfs' (Dwarf Green, *DG*); hybrids between dwarfs and talls (Dwarf x Tall, *DT*); and a tall form of Pacific region origin, acclimatized to Sri Lanka San Ramon, *SR*). Mature nuts selected from well-grown palms, were germinated and raised in a soil and coir dust mix under well-watered conditions, for seven months, as per standard nursery practice at CRISL. Seedlings of uniform size in terms of number of leaves, seedling height and girth, were transferred to 40 litre capacity plastic pots containing sandy loam soil and placed in a plexiglass roofed, open sided, plant house. The soil used was from a *Madampe Series* site (Somasiri *et al.*, 1994), similar to that of the field experiment. Seedlings were uniformly fertilized one month after planting with 10 g of dolomite and 50 g of the CRI Young Palm Fertilizer Mixture (YPM). A Randomized Complete Block Design (RCBD), with 3 replicates, was used for the experiment. There were 8 treatments (4 varieties and two watering regimes *viz.* well-watered and 'droughted' i.e. withholding water for 63 days before watering to field capacity), with 3 plants per plot.

All seedlings were allowed to establish under the well-watered regime for 3 months prior to applying the 'droughted' treatment – a 63-day drought broken by watering to field capacity.

Field experiment

Forty forms or accessions (form/acc) including local and exotic dwarfs, tall forms and hybrids (Table 1), approximately 15 years old, from the germplasm collection at Pottukkulama Research Station, Pallama, Sri Lanka were used for the field experiment.

Table 1. Coconut forms or accessions (abbreviations in parenthesis) used in the field experiment

Variety	Forms or Accessions		
typica (Talls)	Wellawa (*WL*) Pitiyakanda (*PI*) Palugaswewa (*PW*) St. Annes (*SA*) Melsiripura (*MP*) Goluwapokuna (*GP*) San Ramon Green (*SRG*) Navasi (*NV*) Gon Thembili (*GT*) Navasi Thembili (*NT*)	Moorock (*MO*) Namalwatta (*NW*) Ambakelle Special (*AS*) Margaret (*MA*) Mangalaeliya (*ME*) Keenakelle (*KK*) San Ramon Russet (*SRR*) Bodiri (*BD*) Ran Thembili (*RT*)	Debarayaya (*DBY*) Clovis (*CL*) Razeena (*RA*) Kasagala (*KS*) Goyambokka (*GB*) Maliboda (*MB*) Horakelle (*HK*) Walahapitiya (*WP*) Kamandala (*KD*) Porapol (*PP*)
nana (Local Dwarfs)	Dwarf Brown (*DB*) Dwarf Yellow (*DY*)	Dwarf Kundasale (*KUD*)	Dwarf Red (*DR*) Dwarf Green (*DG*)
nana (Exotic Dwarfs)	Cameroon Red Dwarf (*CRD*)	Brazilian Green Dwarf (*BGD*)	
aurantiaca (King coconuts)	King coconut (*KC*)	Rathran Thembili (*RTT*)	
Hybrids	Dwarf Green x Tall (DT)	Dwarf Yellow x Tall (*DYT*)	

All form/acc were planted in single random blocks of at least 30 palms each, using a 7.8 x 7.8 x 7.8 m triangular planting system. The entire

plantation has been fertilized regularly with 3 kg per palm per year of the CRI Adult Palm Fertilizer Mixture (APM) and managed as per practices recommended by CRISL (Liyanage, 1999), from the time of planting. The site was excellent for coconut with a flat terrain and a uniform deep sandy loam Madampe Series soil in Land Suitability Class S 1 (Somasiri *et al.*, 1994).

Six adjacent palms from the centre of each block were monitored over 14 consecutive weeks during January to April 2003; the first 11 weeks were the natural drought period. Although there were some brief showers during the 77-day 'drought' period the rainfall was no greater than 5 mm on any given day. The 77-day drought was broken by natural rainfall.

Chlorophyll fluorescence

In seedlings, the youngest, fully-expanded leaf was selected for measuring chlorophyll fluorescence. In mature palms, it was the ninth leaf (counting from the top) which is representative of the physiologically most active middle whorl (Braconnier and Bonneau, 1998). In tall, adult trees, conducting *in situ* leaf measurements was impractical. Hence, detaching a complete leaflet from the ninth leaf, and dropping it to the ground for rapid measurements was adopted as the standard practice. Previous tests in 3-year old palms showed that determining stable leaf gas exchange values and other physiological parameters on detached leaves was acceptable provided that the measurements are completed within two minutes of detachment (Nainanayake, 2004). The maximum quantum yield of photosystem II, given by the dark-adapted F_v/F_m (the ratio of the variable to the maximum chlorophyll fluorescence), was measured using a fluorometer (PEA Mark 2, Hansatech Instruments Ltd., Kings Lynn, UK).

A preliminary experiment was conducted on *AS* seedlings to determine the minimum period required for dark adaptation, in preparation for measuring the dark-adapted F_v/F_m. A portion of the youngest fully expanded leaf was covered by a clip provided with the instrument, and allowed to remain for 2 minutes before recording the F_v/F_m value. Recordings were repeated, increasing the time allowed for dark adaptation by 2 minutes, up to 10 minutes dark adaptation; and then increasing by 5 minutes up to 30 minutes. Measurements were made on both well-watered and droughted seedlings; six of each category.

Physiological parameters

The rate of photosynthesis (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}) and the rate of transpiration (E, mmol m^{-2} s^{-1}) were measured using gas exchange technique in a closed-system portable photosynthesis system (Li-6200, LI-COR Inc., USA) and the instantaneous water use efficiency (ω_{inst}, μmol CO_2 mol^{-1} H_2O) was calculated as the ratio of A/E. The rate of photosynthesis observed at the end of the drought is termed minimum rate of photosynthesis (A_{min}). The leaves selected for these measurements were the same as for chlorophyll fluorescence.

Soil moisture content

In the plant house experiment, mean soil moisture content (θ %) was determined gravimetrically on approximately 5 g samples of soil taken at a depth of 45 cm, through a small access tube in each pot. Soil samples were drawn at the time of fluorescence measurements.

In the field experiment, the mean gravimetric soil moisture content of the top 150 cm (θ_{mean} %), where the highest concentration of coconut roots are found (Vidhana Arachchi, 1998), was determined at 14–day intervals, during the drought period. Soil core samples of approximately 200 cm^3 were drawn from two points in each form/acc plot, 1 m away from the base of two palms, and at 50 cm, 100 cm, and 150 cm depths. θ values at different depths *viz.* 50 cm, 100 cm, and 150 cm (θ_{50}, θ_{100}, θ_{150}) were determined gravimetrically. The average of the means at the 3 depths, at both sampling points, was taken as the plot mean θ (θ_{mean}), and as a measure of its soil moisture status. The θ_{mean} was assumed to be applicable to the other palms in the form/acc plot, as all six palms were adjacent to each other and in the middle of the form/acc block.

Data analyses

The software package, Statistical Analysis System (SAS Release 8.2, SAS Institute Inc., USA) was used in statistical analyses mainly for Analysis of Variance (ANOVA).

RESULTS

Although the plant house was well ventilated with 3 large exhaust fans, the air temperature ranged from 29°C to 45°C during the day and 23°C to 29°C during the night. Relative humidity ranged from 65% to 75% during the

day and 75% to 85% at night. Light penetration through the roof was appreciable, and the incident photosynthetic PPFD was up to 950 µmol m^{-2} s^{-1}. At the time leaf measurements were taken, between 09:30 and 12:00 hrs, PPFD was between 600 and 950 µmol m^{-2} s^{-1}. According to Madurapperuma *et al.*, 2002, the diurnal time course of leaf gas exchange in coconut is more or less constant between 10 and 13h.

Soil moisture content

In the plant house experiment, the mean soil moisture content (averaged over form/acc) in the well-watered pots was steady over the trial period, ranging from 37.4% to 38.6% (Fig. 2a). In the droughted pots however, θ declined to about 4.5% at the end of the imposed 63-day drought. There were substantial differences in the moisture depletion patterns of the form/acc (Fig. 2b). Soil moisture depletion in the *DT* and *SR* pots was more rapid than in the *DG* and *AS* pots. This resulted in low θ in pots with *DT* and *SR* at the end of dry period (3.3% and 2.5% respectively) compared to *DG* and *AS* (6.6% and 5.6% respectively).

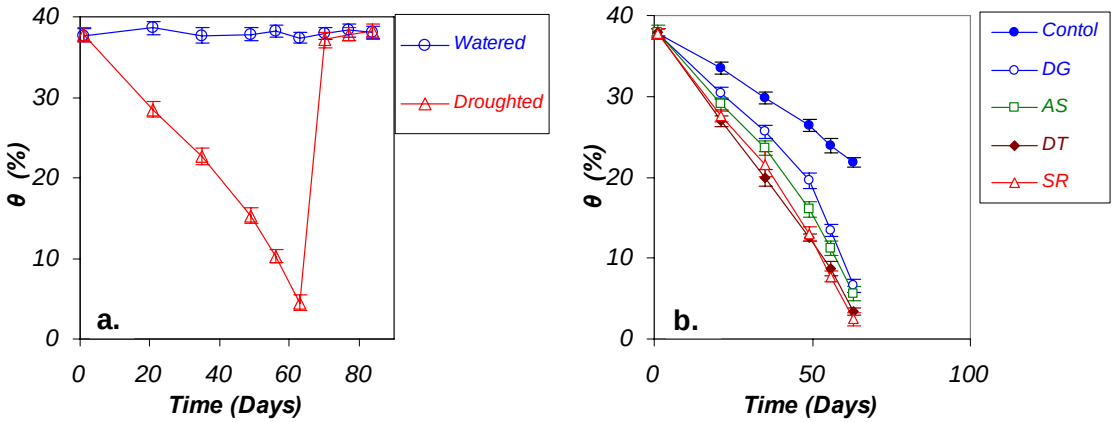

Fig. 2 Mean soil moisture content (θ, %) in pots (a) averaged over the four form/acc in the watered and droughted treatments, and (b) of each form/acc over the 63-day drought period.
(Control – No plant, only soil, DG – Dwarf Green, AS – Ambakelle Special, DT – Dwarf x Tall, SR – San Ramon; SE of the mean is indicated by vertical bars).

In the field trial, all coconut palms were exposed to a 77-day natural drought from January to March 2003. At the onset of the drought, the θ_{mean} averaged 33.4% over all form/acc; the difference between form/acc was small, only about 2.0%. Although the initial rates of decline were similar

(barring few exceptions) there were substantial differences in θ_{mean} among form/acc towards the latter part of the drought (Fig. 3). Differences in the rate of decline were reflected in the minimum soil moisture (θ_{min}) recorded for form/acc (Fig. 4) where *SRR*, *SRG*, *DT* and *DYT* recorded ≤ 8.0% at the end of the drought. *DBY, KS, CRD* and *KC* took up less water and recorded a $\theta_{min,}$ as high as 16.0% while the rest ranged between 11.0 and 14.0%. Generally, across all form/acc, the reduction of θ_{mean} was about a 60% reflecting the severity of the drought.

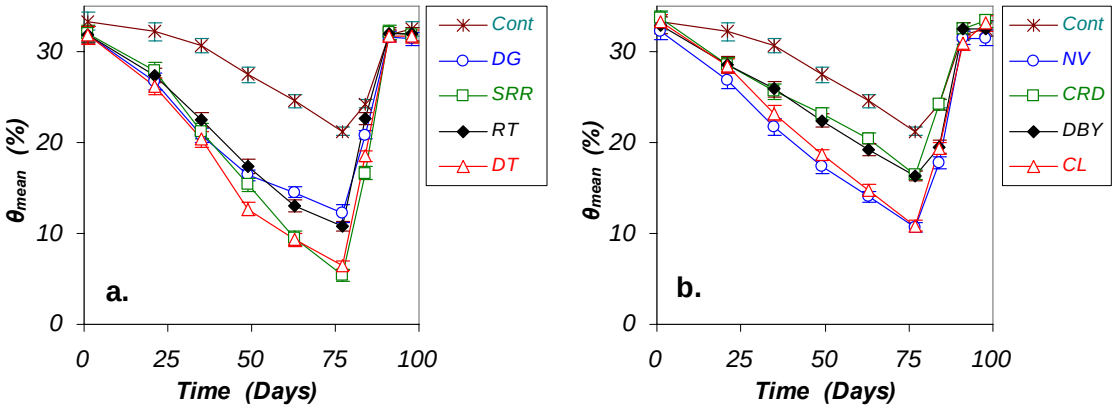

Fig 3. Time course of soil moisture content (θ_{mean} %) in adult palms of eight selected form/acc during the 77-day drought period.

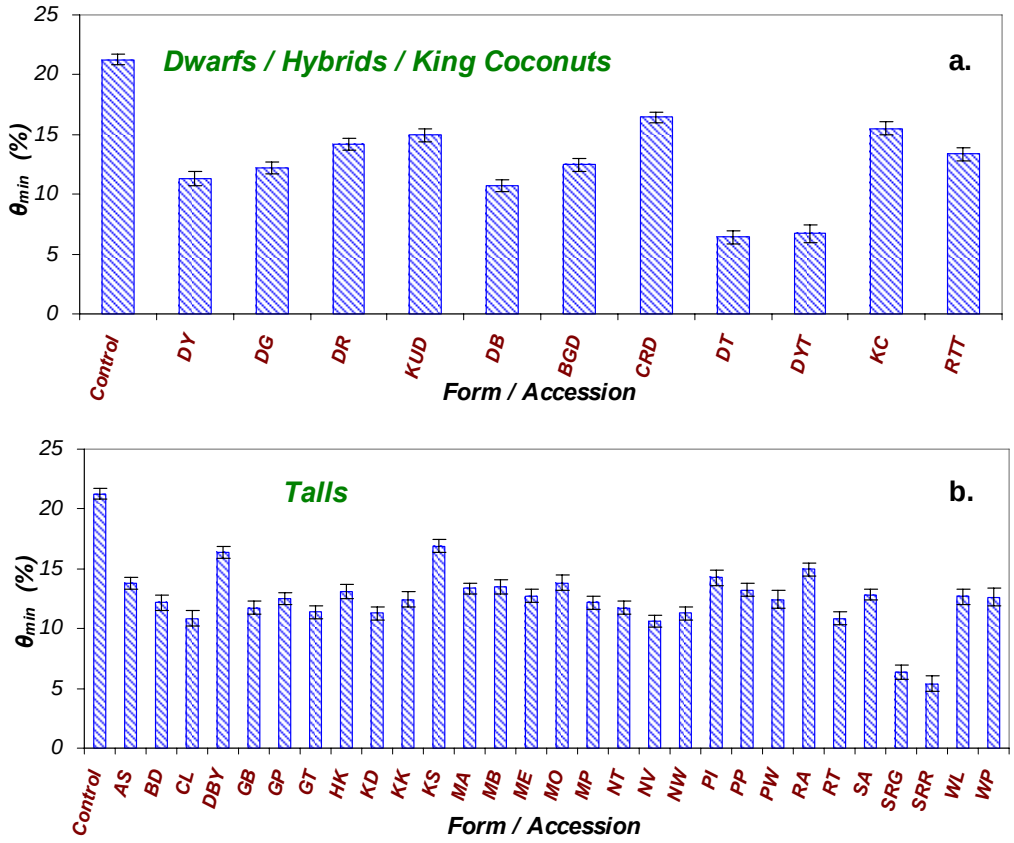

Fig. 4. The minimum soil moisture content in the top 150 cm (θ_{min} %) at the end of the drought, in the plots with (a) dwarf form/acc *DY* to *CRD*, hybrids *DT* and *DYT* and king coconuts *KC* and *RTT* and (b) Tall form/acc *AS* to *WP*.

The experiment was sited on a block of land which was quite uniform and with a flat terrain. Two bare plots, with no palms, were also included to serve as 'controls'. The θ_{mean} of these plots were 11.2 and 12.9%. It appears that soil water loss by evaporation in the block is quite uniform, and therefore substantial differences in θ_{mean} at the end of the drought could be ascribed to differences in the quantum of water absorbed and/or lost by transpiration by the form/acc concerned.

Maximum quantum yield of photo system II (F_v/F_m)

In preliminary investigations, F_v/F_m reached a constant maximum value after a dark-adaptation period of 20 minutes, in both well-watered and droughted coconut seedlings (Fig. 5a and 5b). Hence, a minimum of 20 minutes dark-adaptation was used for subsequent measurements. In adult palms, F_v/F_m was measured on detached leaflets of the 9th leaf and the dark-adaptation was achieved by placing the leaflets in a black polythene bag for 20 minutes before measuring.

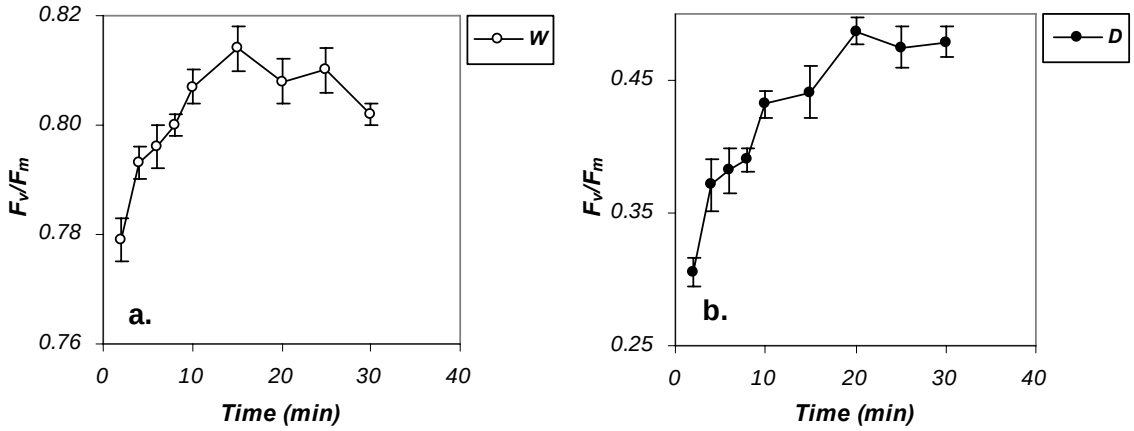

Fig. 5. Effect of duration of dark adaptation on the mean F_v/F_m of the youngest fully expanded leaf of 9-month old (a) well-watered and (b) droughted coconut seedlings grown in pots in a plant house (n = 6)

In the plant house experiment, well-watered *DT* seedlings recorded slightly higher (4%) dark-adapted F_v/F_m values than the other three form/acc which, were not significantly different (Fig. 6a). In the droughted treatment, in all form/acc, the reduction of F_v/F_m, was marginal up to day 35 of the drought, but declined sharply after day 49 (Fig. 6b). During this sharp decline phase, *SR* maintained a marginally higher value of F_v/F_m; there was no difference between other form/acc was observed at the end of the drought. The recovery with re-watering was generally slow with *SR* slightly ahead of the others (Fig. 6b).

When F_v/F_m was plotted against corresponding θ the patterns were similar but the differences more conspicuous; *SR* maintained markedly higher F_v/F_m ratios at lower levels of θ (Fig. 6c). At a θ of 10%, *SR* recorded the

highest F_v/F_m of 0.71 while *DT, AS* and *DG* recorded 0.66, 0.59 and 0.52 for respectively.

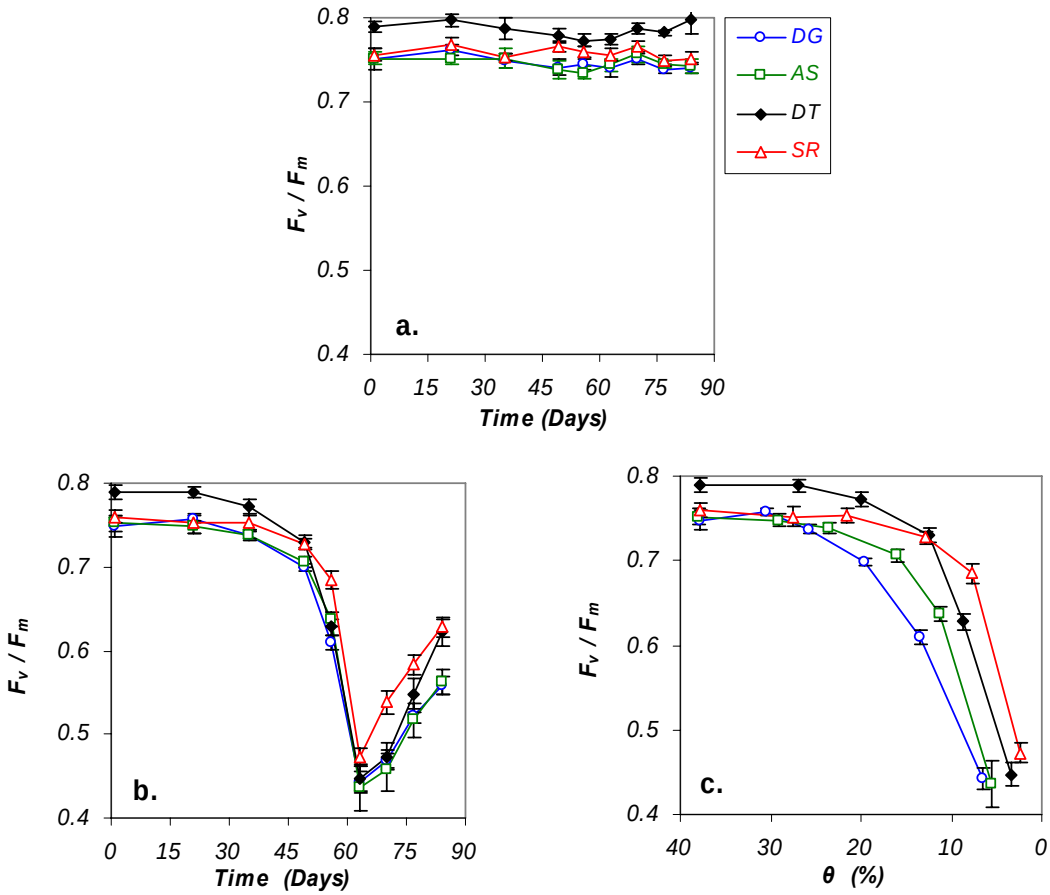

Fig. 6. Mean ratio of F_v/F_m of coconut seedlings in the well-watered and droughted treatments (a and b respectively). F_v/F_m plotted against the corresponding soil moisture content (θ, %) in the drought phase is at c.

In the case of adult palms, rain fed but with no moisture stress, there were no significant differences in dark adapted F_v/F_m values (averaging 0.756) between form/acc (data not shown). With the onset of the drought, although there were no major changes in the F_v/F_m values up to day 14, most form/acc showed a slight decline from day 28 followed by a sharp decline after day 42 (Fig. 7 shows four selected form/acc with contrasting behaviour).

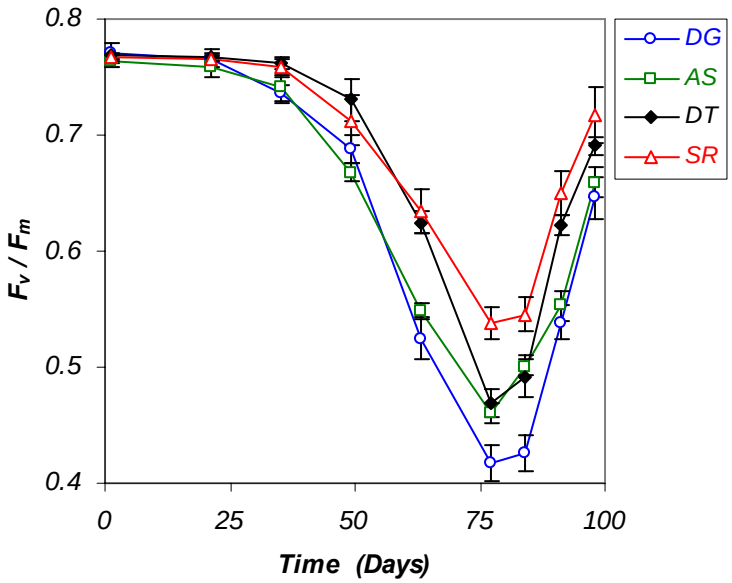

Fig. 7. Mean F_v/F_m of four selected form/acc of adult coconut palms over the 77-day drought period and the period followed by a 21-day rainy period.

The minimum F_v/F_m at the end of the drought was significantly higher (> 0.5) in eight form/acc (*SRR, SRG, SA, DBY, KS, CL, PI & PW*) the rest; the lowest values (< 0.4) were recorded in *BD*, *HK*, *KK* and *KD* (Fig. 8). The high F_v/F_m group of form/acc appears to have been able to protect the integrity of the thylakoid membranes of the photosystem II and thereby maintain the rate of photosynthesis with little or no change. This indicates relatively greater drought tolerance. In fact, A_{min} (rate of photosynthesis at the end of the drought) of these form/acc more or less matched the ranking based on the maintenance of high photosynthetic rates. For example, *SRR, SRG*, *CL*, *SA* and *PI* grouped together had a significantly higher A_{min} than the rest (Fig. 9). The only exceptions were *DBY* and *KS* which maintained a high F_v/F_m at the end of the drought but with substantially lower rates of photosynthesis than the rest. Grouping based on the water use efficiency was more or less similar to that of A_{min} (data not shown). Most form/acc were slow to recover and their F_v/F_m ratios were 10-25% less than the initial values even 3 weeks into the wet period. However, *SRR, SRG, DBY* and *KS* recovered well; their F_v/F_m were only about 8% less than the initial F_v/F_m. Therefore, in general, the screening for drought tolerance based on yield related physiological parameters such as the rate of photosynthesis and/or water use efficiency matches well screening based on chlorophyll fluorescence.

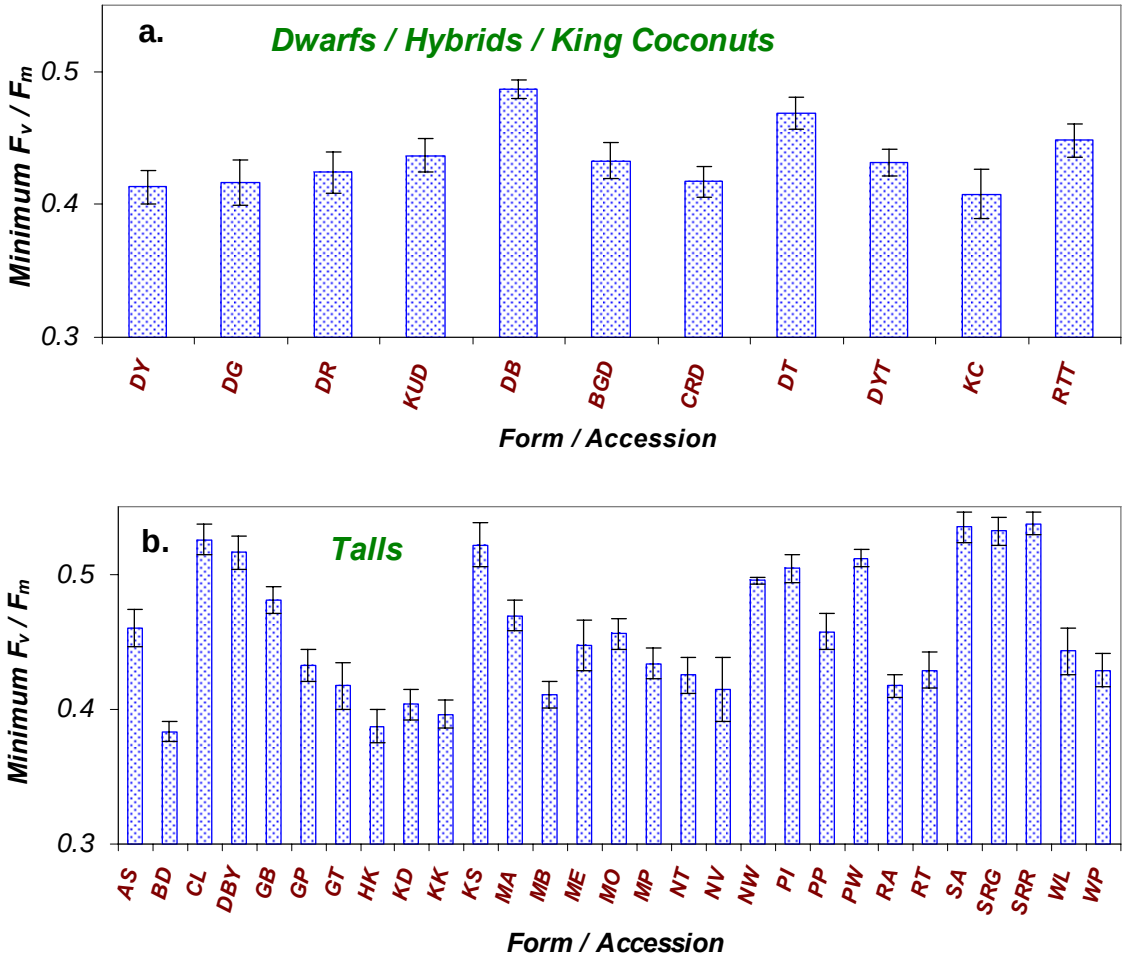

Fig. 8. Minimum F_v/F_m of adult coconut form/acc at the end of the 77-day drought period.

Seedlings versus adult palms

The general pattern of decline in the F_v/F_m values during the course of the drought was similar in seedlings and adult palms (Fig. 10). However, in the case of seedlings the decline was somewhat steeper than in adult palms. This may be ascribed to the moisture in the relatively small mass of soil in the pots being rapidly depleted in contrast to the much larger mass of soil and soil moisture available to adult palms in the field.

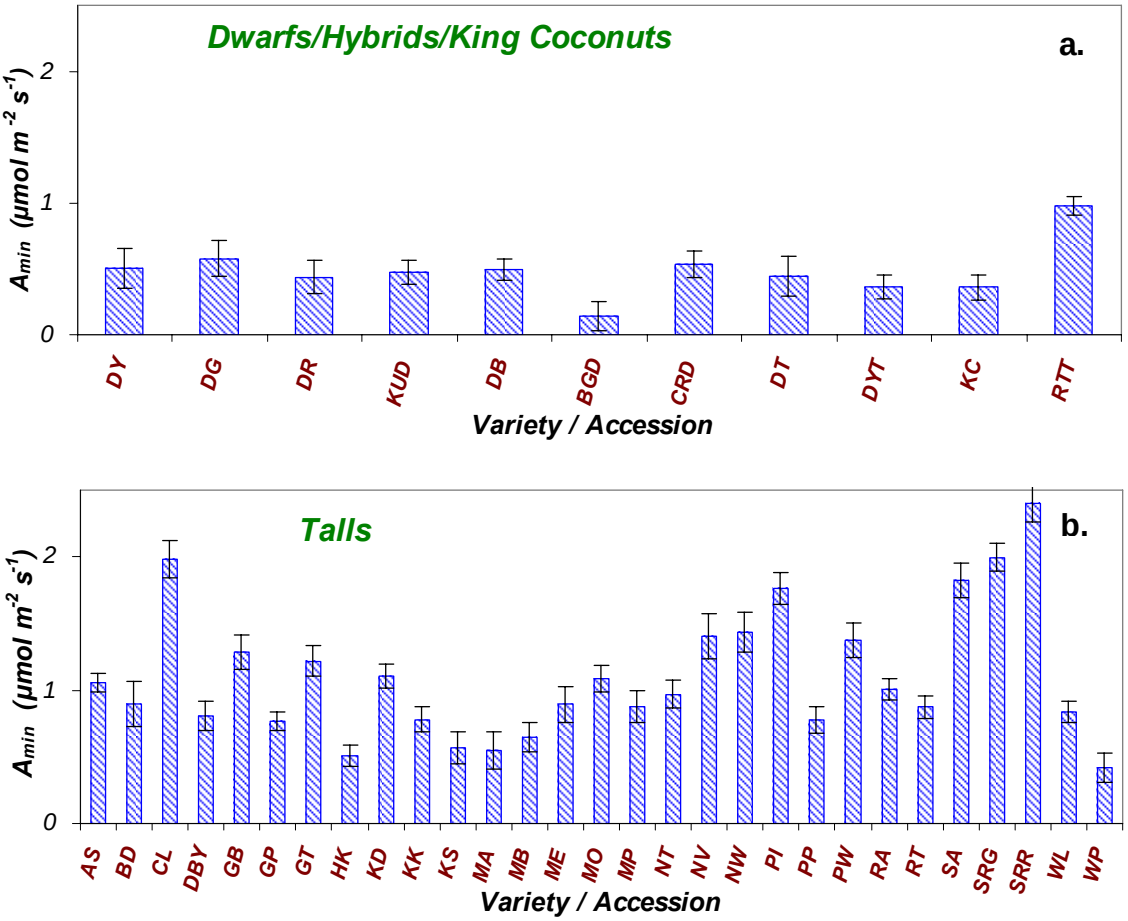

Fig. 9. The rate of photosynthesis in adult palms at the end of the drought (A_{min}, µmol m^{-2} s^{-1}) for different form/acc of coconut.

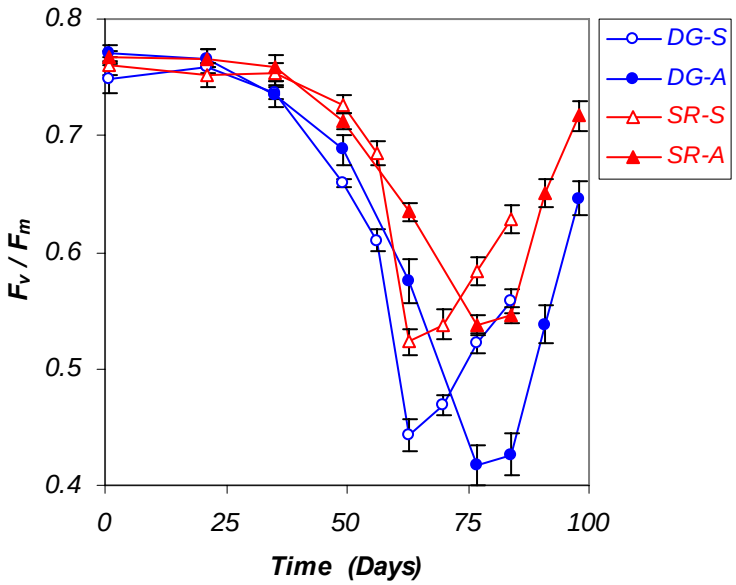

Fig.10. Mean F_v/F_m of coconut seedlings (*S*) and adult palms (*A*), of two selected form/acc (*DG and SR*), over the 63-day and 77-day drought periods, respectively, and the 21-day wet periods (*S and A indicate seedlings and adult palms respectively*).

DISCUSSION

Chlorophyll fluorescence versus yield as a parameter for screening

Plant breeders are interested in testing morphological and physiological traits, as reference parameters to screen crop species for putative drought tolerance, instead of the most integrative, comprehensive and heavily used parameter, yield. The compelling factors here are the desire to save time and the difficulty in evaluating the effect of a particular dry spell on the yield of a perennial tree crop such as coconut. Coconut has a long generation period of about 15 years from seed nut to a bearing palm with a stable yield. It takes a year from the opening of the inflorescence to produce mature nuts. This visible cycle is preceded by a 32-month non-visible cycle of development from flower primodia up to the opening of the inflorescence - a development cycle of 44 months in all. This complexity and lengthy generation period makes it very difficult to separate the effects of drought on coconut yields. Button nut setting, their growth, development of nut water and kernel occurs over different periods during the year-long visible maturation cycle. The extent of adverse drought effects such as immature nut fall, reduced nut size or poorly developed kernel will

therefore depend on the stage of nut development exposed to the drought. To add to the complexity: nut development stages are sequential in a single inflorescence, but they overlap in the palm and in the field. Droughts could also impact on different stages of the non-visible development cycle. And drought tolerance evaluation based on yield is a very long-term exercise. Therefore, the development of a rapid, reliable and less invasive technique/s such as chlorophyll fluorescence appears important and advantageous in the context of screening for drought tolerance.

There is a long standing controversy in regard to using parameters, other than yield, to screen for drought tolerance. Lawlor (1995) argued that there is no direct relationship between physiological parameters such as rate of photosynthesis and crop yield. However, evidence to the contrary has emerged in the recent past. For instance, Mann (1999) reported a good correlation of increasing rate of photosynthesis with higher crop yields while Mitchell *et al.*, (1999) found a 50% average increase of photosynthesis in a single genotype of spring wheat grown at seven sites in Europe (Germany, UK, Ireland, Belgium and The Netherlands) which increased grain yield by an average of 35%. Ainsworth *et al.*, (2002) reported comparable increases in photosynthesis and yield in soybean, under elevated CO_2. Thus the use of parameters, other than yield, to screen crops is becoming more and more acceptable with evidence on different crops from different parts of the world. In this context, chlorophyll fluorescence emerged as a quick, non invasive tool to investigate the activity of photosystem II of the photosynthetic machinery in the plant leaf. This method has been heavily used in the recent past to evaluate the impact of stress conditions on the photosynthetic machinery and consequently crop productivity under given conditions.

Applicability of chlorophyll fluoresence in coconut and other crops

Chlorophyll fluorescence works on the principle that photosynthesis is one of the core functions in the physiology of plants. The functional state of photosynthesis has been considered an ideal physiological activity to monitor the health and vitality of plants (Clark *et al*., 2000). The health or the integrity of the internal apparatus (thylakoid membrane) drives the photosynthetic process within a leaf. This integrity which can be assessed by chlorophyll fluorescence provides a rapid and accurate means of detecting and quantifying the plant's tolerance to stress. Moreover, previous researches indicate that chlorophyll fluorescence values of detached leaves strongly correlate with plant mortality due to environmental stresses such as freezing, heat chilling and salinity (Smillie

and Hetherington, 1983; Greaves and Wilson, 1987; Brennan and Jefferies, 1990; Yamada *et al.*, 1996; Hakam *et al.*, 2000). It permits quantification of species or varietal tolerance with limited whole plant experiments. Using F_v/F_m values Percival and Sheriffs (2002) selected 30 woody plant species with the ability to maintain photochemical efficiency even after a 70-day drought. The ranking by visual observation was similar to that based on the rate of photosynthesis. As photosynthesis is the primary physiological function that contributes to the productivity of a plant, any species identified as drought tolerant based on F_v/F_m may reflect this tolerance in their yield or productivity parameters.

Adopting a similar approach to evaluate adult coconut form/acc in the present experiment, it was possible to group 6 form/acc (*SRR, SRG, SA, CL PI & PW*) as drought tolerant based on high readings for both F_v/F_m and the rate of *A* observed at the end of the drought. The exceptions observed were *DBY* and *KS* which maintained a high F_v/F_m at the end of the drought but with relatively low rates of photosynthesis. Stomatal conductance (g_s) of these two form/acc revealed a probable cause for this discrepancy. Their g_s decreased markedly at a fairly early stage of the drought, when θ_{mean} was around 25%, indicative of an early response to conserve water. Restriction of gaseous exchange at stomatal level appears to be the primary cause for the rapid reduction of *A* observed in *KS* and *DBY*. These results point to the possibility of using F_v/F_m as a promising tool to screen coconut palms for their drought tolerance. Concurrent monitoring of the gas exchange parameter would be useful not only to provide confirmation but also to identify the exceptions.

Consistency of past selections with present findings

KS and *DBY* had been originally selected for inclusion in the germplasm conservation pool because their parent populations had withstood severe droughts. Hence they were also expected to possess a degree of drought tolerance. Closure of stomata from the very early stages of the drought is a likely adaptation to survive long and frequent dry spells. *SRR, SRG* and *CL* were of common origin from the Pacific region, probably from the Philippines and introduced to Sri Lanka few decades ago. Frequent changes in climate in the Pacific region may have contributed to their adaptation to withstand a wide range of stressful environments. Some inherent characteristics of these form/acc such as a thick kernel may have contributed to the survival of developing seedlings under adverse weather conditions. The common origin of these three form/acc and the *CL* being an open-pollinated population of the variety San Ramon was recently been

confirmed with evidence from molecular studies (Perera, 2005). *BD*, *HK*, *KK* and *KD* which appeared to be sensitive to drought may have evolved in good, deep soils with higher water holding capacity and/or in the wetter parts of the intermediate zones of the coconut growing areas. Therefore, most of the form/acc selected for the CRI germplasm collection, based on different morphological or other general characteristics, show some degree of consistency with the present evaluation based on the chlorophyll fluorescence. Consistency of grouping patterns based on F_v/F_m, the rate of photosynthesis and water use efficiency provides a firm basis for using chlorophyll fluorescence as a selection criterion. Therefore, identifying putative drought tolerant coconut form/acc based on the rapid, non invasive tool of chlorophyll fluorescence is a distinct possibilty. The similarity observed in the performance of seedlings and adult palms is a great advantage. This enables the identification of inherent capacities and potentials at the seedling stage thus making a huge saving in the time requirement for evaluating coconut varieties.

The preliminary findings clearly demonstrate the potential of chlorophyll fluorescence. Nevertheless, the fluorometer used for these measurements was a basic model that was not equipped with a modulated light system that would have enabled a full range of measurements, as discussed in the introductory sections. A more comprehensive evaluation of the coconut germplasm collections should therefore be conducted with the full spectrum of fluorescence parameters under natural light conditions. It is also important to re-evaluate and confirm the above findings using long-term yield data so as to fully validate the use of this rapid and convenient selection technique.

CONCLUSIONS

Chlorophyll fluorescence which has been widely used for screening many crops and plant species for drought tolerance appears to be also applicable to coconut. The maximum quantum yield or the efficiency of PSII, as indicated by the dark-adapted F_v/F_m, is a promising, non-invasive parameter for screening coconut germplasm. Using this approach, *SRR, SRG, SA, CL PI & PW* were the most drought tolerant of the 40 genotypes tested. The results generated by using F_v/F_m, and other important, yield-related physiological parameters such as the rate of photosynthesis and water use efficiency, were consistent. This provides a firm basis for using leaf chlorophyll fluorescence as a selection criterion. The great similarities observed between seedlings and adult palms with respect to fluorescence parameters is a key finding that enables fairly confident predictions on the

performance of adult palms, based on studies with seedlings. A detailed evaluation of the CRI germplasm collections using the full spectrum of chlorophyll fluorescence parameters, and relating the results to long-term yield data, is important to make a strong case for using the technique in coconut.

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